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Chlamydial infections of the cervix

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A major problem in the control of non-specific genital infection (NSGI) is the difficulty of identifying the disease in women. In men, non-specific urethritis (NSU) is diagnosed when there are significant numbers of polymorphonuclear leucocytes in urethral secretions, certain known causes of urethritis such as *N. gonorrhoeae* having been excluded. This approach is not possible in women. Polymorphonuclear leucocytes are present in the lower genital tract in many women, so that their enumeration has little diagnostic significance, and the value of clinical or cytological evidence of cervical inflammation is unproved.

The only women likely to be suspected of having NSGI are those who have sexual contacts with NSU, and these women may receive a course of treatment; women who are unaware that they have a sexual contact who has NSU are likely to remain undiagnosed. The identification and treatment of women with NSGI will be difficult until the infecting organism or organisms can be demonstrated.

Evidence has now accumulated which indicates that organisms of *Chlamydia* Group A may cause many infections of NSU. By the use of a sensitive cell culture system it has been shown that *Chlamydia* can be isolated from about 40 per cent. of men with NSU (Dunlop, Vaughan-Jackson, Darougar, and Jones, 1972; Oriel, Reeve, Powis, Miller, and Nicol, 1972). The organisms have also been found in 25 to 30 per cent. of men with gonococcal urethritis, and these men may be particularly liable to develop post-gonococcal urethritis (Richmond, Hilton, and Clarke, 1972; Powis, Oriel, and Reeve, 1973). *Chlamydia* is rarely found in men who do not have urethritis (Oriel and others, 1972). Some recent observations indicate that chlamydial infection of the urethra may be accompanied by significant, and sometimes rising, titres of chlamydial antibodies, detected by immunofluorescent and radio-immune precipitation techniques (Reeve, Gerloff, Casper, Philip, Oriel, and Powis, 1974). Although direct proof cannot be obtained in the absence of experiments using human volunteers, most observers believe that *Chlamydia* are pathogenic in the male urethra. Women who are sexual contacts of men with NSU often harbour the organisms in the cervix. Primary (source) contacts of these men almost invariably have a demonstrable chlamydial infection; secondary contacts are less often infected (Oriel and others, 1972). Alexander (1973) has observed that many women with chlamydial infection show clinical evidence of cervical inflammation, although Dunlop, Jones, and Al-Hussaini (1964) believe that a characteristic sign is the presence of follicles and other fine changes in cervical structure visible only with the operating microscope. Like men, women with genital chlamydial infection often show an immunological response (Reeve and others, 1974). If *Chlamydia* is accepted as a cause of NSU, its identification in a woman might be taken to indicate that she has NSGI requiring treatment.

Little is known of the incidence of chlamydial infection of the female genital tract. Holt, Pedersen, Wang, Kenny, Foy, and Grayston (1967), using the yolk-sac technique, obtained *Chlamydia* from six of 42 cervical specimens from women attending a venereal disease clinic in Seattle, but Ford and McCandlish (1971), using the same technique, failed to isolate *Chlamydia* from the cervix in 44 unselected women, 22 of whom, being the inmates of a gaol, were assumed to be fairly promiscuous. The yolk-sac technique, however, is now known to be less sensitive than cell culture for *Chlamydia* isolation (Gordon, Dressler, and Quan, 1967; Gordon, Harper, Quan, Treharne, Dwyer, and Garland, 1969). Other studies have been made of special groups: women in whom chlamydial ocular disease was known to be present or who produced offspring with chlamydial ocular disease (Dunlop and others, 1964; Dunlop, Freedman, Garland, Harper, Jones, Race, du Toit, and Treharne, 1967), and women who were known to be sexual contacts of men with NSU (Dunlop and others, 1972; Oriel and others, 1972). The incidence of genital chlamydial infection in other groups is not known.

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As the first of several such studies, we are reporting here the investigation of a group of women who were attending a department of sexually-transmitted diseases (STD) for the first time. In addition to conventional microbiological tests, *Chlamydia* isolations from the cervix were attempted in every case, and the results correlated with the case history, clinical evidence of infection, and the presence of other STD.

Material and methods

The patients were seen in the Department of Venereal Diseases, St. Thomas' Hospital. During November and December, 1972, all women who were attending the Department for the first time were included in the survey. After the history had been taken, patients were examined in the lithotomy position. Specimens were taken from the urethra, vagina, and cervix for the identification of *N. gonorrhoeae*, *T. vaginalis*, and *C. albicans* by conventional methods. An Ayre speculum was used to take material for cervical cytology. A specimen for *Chlamydia* culture was collected from within the cervical canal with a cotton-wool swab and incubated after centrifugation on to irradiated McCoy cells. Details of these clinical and microbiological procedures have been recorded elsewhere (Oriel and others, 1972).

The criteria for a cytological diagnosis of cervical inflammation were the presence of both inflammatory cells and epithelial changes of Papanicolaou Grade II (Hughes and Dodds, 1968).

Patients were assigned to appropriate diagnostic categories according to the following criteria:

Gonorrhoea Characteristic intracellular Gram-negative diplococci on microscopy and/or positive cultures and/or positive fluorescent tests.

Trichomoniasis Characteristic motile organisms on a wet slide and/or positive cultures.

Candidosis Characteristic yeast cells or mycelium on a Gram-stained slide and/or isolation of *C. albicans* on culture.

Genital warts Clinical appearance.

Genital herpes Clinical appearance, usually confirmed by cytopathic effect on tissue culture.

NSU contacts Women who had had intercourse within the preceding 8 weeks with men in whom the diagnosis of NSU had been made in our own or in another similar department. These women were subdivided into primary (source) and secondary contacts, and into contacts of men with first attacks of NSU and of men with recurrent NSU.

Gonorrhoea contacts Women who had had intercourse with men who were similarly known to have had gonorrhoea within the preceding 8 weeks.

Negative history and negative routine tests Women with no history of recent sexual contact with men with gonorrhoea or NSU, and who showed no clinical evidence of genital warts or herpes and no microbiological evidence of gonorrhoea, trichomoniasis, or candidosis.

Results

During the investigation 318 women were studied. The youngest patient was aged 15 years and the oldest 45 years (mean 24·4 ± S.D. 6·5). Their ethnic origin was: Asiatic, 8 (2·5 per cent.); Negro, 73 (23 per cent.); Caucasian 237 (74·5 per cent.). The reasons for the patients' attendances were: 153 women because they were sexual contacts of men with STD; of these women, 105 were asymptomatic. A further 165 women were not known to be contacts of such men, but came of their own accord, or were referred by their doctors, for the exclusion of STD; of these women, 45 were asymptomatic.

A past history of STD, treated elsewhere, was given by 49 women; a history of gonorrhoea in ten, of trichomoniasis in five, candidosis in nineteen, genital warts in 2, genital herpes in four, and nine had previously been treated as contacts of men with NSU. As regards contraception, 128 women were taking an oral contraceptive, 22 used an intra-uterine device, six a cervical cap, and one a spermicidal foam; the male partners of a further 24 used a condom, but not invariably; no contraceptive precautions were taken by 124 women, and an additional thirteen patients were pregnant. During the 4 weeks before attendance, 293 of the 318 women admitted intercourse, 52 of them with more than one man.

DIAGNOSTIC CATEGORIES

From the history, clinical examination and results of routine microbiology, patients were assigned to diagnostic categories as shown in Table I. It will be seen that 89 of the 318 patients (28 per cent.) came to the department because they were sexual contacts of men with NSU, which again emphasizes how common the disease is.

CHLAMYDIA ISOLATION

The results of culture for *Chlamydia* in the 318 patients are shown in Table II. Contamination by bacteria or yeasts, making the preparation impossible to interpret, occurred in 71 specimens (22 per cent.). Of the uncontaminated cultures, 45 of 247 (18 per cent.) yielded chlamydial isolates.

The 45 *Chlamydia*-positive women were between the ages of 17 and 34 years, their mean age being 22·8 ± S.D. 4·8 years. None was Asiatic, 7 were Negro, and 38 Caucasian. Of these 45 women, 32 attended because they were contacts of men with STD; of these 32 women, eighteen were asymptomatic. A further thirteen women were not known to be contacts of such men, and of these, two were asymptomatic. Thus, twenty of the 45 *Chlamydia*-

positive women (44 per cent.) were symptom-free. A past history of STD was given by four women: one had had genital warts, and three had been treated as contacts of men with NSU.

An oral contraceptive was being taken by 27 of the 45 *Chlamydia*-positive women (60 per cent.); among the 202 *Chlamydia*-negative women, 75 (37 per cent.) were taking an oral contraceptive, but the difference is not statistically significant ($\chi^2 = 0{\cdot}96$; $P > 5$ per cent.). Among the remaining *Chlamydia*-positive women, none used an intra-uterine device, but one used a cervical cap, and the contacts of two more used condoms. No contraceptive precautions were taken by thirteen women, and a further two were pregnant. During the 4 weeks before attendance, 43 of the 45 *Chlamydia*-positive women had had intercourse, eleven of them with more than one man.

The relationship between the results of culture for *Chlamydia* and the patients' diagnostic categories is shown in Table III. Most of the positive results were seen in contacts of men with NSU and in women with gonorrhoea; of those providing valid specimens, seventeen of 51 patients who were contacts of men with first attacks of NSU were *Chlamydia*-positive (33 per cent.); three of 22 contacts of men with recurrent NSU were *Chlamydia*-positive (14 per cent.); nine of 28 patients with gonorrhoea (32 per cent.) and seven of twenty patients with gonorrhoea and trichomoniasis (35 per cent.) were *Chlamydia*-positive.

TABLE I *Diagnostic categories of* 318 *patients*

Diagnostic category	*No. of patients*
Gonorrhoea	39
Gonorrhoea plus trichomoniasis	27
Trichomoniasis	32
Candidosis only[a]	44
Genital herpes	4
Genital warts	13
Non-specific salpingitis	1
Gonorrhoea contacts (*N. gonorrhoeae* tests negative)	13
NSU contacts	
Of men with first attacks of NSU	55
Of men with recurrent NSU	29
NSU contacts with trichomoniasis	5
Negative history and negative routine tests	56
Total	318

[a] Five patients with gonorrhoea, six with trichomoniasis, two with genital herpes, four with genital warts, and twelve NSU contacts also had candidosis.

TABLE II Chlamydia *isolates from* 318 *unselected women*

Number of specimens taken	318
Number *Chlamydia*-positive	45
Number *Chlamydia*-negative	202
Specimens contaminated	71

TABLE III Chlamydia *isolation and diagnostic category*

Diagnostic category	Chlamydia *culture*			*Per cent.* Chlamydia *positive*
	Positive	*Negative*	*Contaminated*	
Gonorrhoea	9	19	11	32
Gonorrhoea plus trichomoniasis	7	13	7	35
Trichomoniasis	1	24	7	4
Candidosis	1	17	26	6
Genital herpes	1	2	1	
Genital warts	2	10	1	
'Non-specific' salpingitis	0	1	0	
Gonorrhoea contacts (N. gonorrhoeae not isolated)	3	10	0	
NSU contacts				
Of men with first attacks of NSU	17	34	4	33
Of men with recurrent NSU	3	19	7	14
NSU contacts with trichomoniasis	0	5	0	
Negative history negative routine tests	1	48	7	2
Total	45	202	71	18

The results among patients who were NSU contacts varied according to whether the women were primary or secondary contacts; in some instances it was impossible to decide from the history which type of contact she was (Table IV, overleaf). Of primary contacts, eight of thirteen were *Chlamydia*-positive, whereas six of 24 secondary contacts were positive.

Although most commonly seen in NSU contacts and women with gonorrhoea, chlamydial isolates were not confined to these groups, and were observed

TABLE IV Chlamydia *isolations in contacts of men with first attacks of NSU*
(Contaminated specimens excluded)

Type of contact	Chlamydia-*positive*	Chlamydia-*negative*
1°	8	5
2°	6	18
Either 1° or 2°	3	11
Total	17	34

in other diagnostic categories, including trichomoniasis, candidosis, genital herpes, and genital warts. However, only one patient of 49 with negative history and negative routine tests was *Chlamydia*-positive.

ABNORMALITIES OF THE CERVIX

Women with gonorrhoea and trichomoniasis often show clinical and cytological abnormalities of the cervix because of these infections. Excluding these women, the clinical and cytological condition of the cervix in both *Chlamydia*-positive and *Chlamydia*-negative women is compared in Table V. A cervical erosion, Nabothian follicles, and muco-purulent or purulent cervical discharge, alone or in combination, were taken to indicate an abnormal cervix. The results of cervical cytology are also recorded; these were not available for two *Chlamydia*-positive and sixteen *Chlamydia*-negative women.

TABLE V *Changes in cervix in* Chlamydia-*positive and negative women*
(Patients with gonorrhoea and trichomoniasis excluded)

Cervix	Chlamydia	
	Positive	*Negative*
Normal clinically Normal cytology	3	52
Normal clinically Inflammatory cytology	3	29
Abnormal clinically Normal cytology	11	25
Abnormal clinically Inflammatory cytology	9	19
Total	26	125

Abnormalities of the cervix, clinical and/or cytological, were seen in 23 of the 26 *Chlamydia*-positive women without gonorrhoea or trichomoniasis (89 per cent.). Among *Chlamydia*-negative women, abnormalities were seen in 73 of 125 (58 per cent.). The numbers in the *Chlamydia*-positive group were too small to allow an adequate statistical comparison.

Discussion

Chlamydial infection of the cervix was not uncommon in these patients, who were attending our STD Clinic for the first time; 45 of 247 uncontaminated specimens (18 per cent.) yielded isolates. Of these 45 women, twenty were contacts of men with NSU, sixteen had gonorrhoea, and a further three were contacts of men with gonorrhoea. The finding of *Chlamydia* in these patients was not unexpected, since in men chlamydial infection of the urethra is almost confined to those with NSU or gonorrhoea. However, six of the 45 *Chlamydia*-positive women were in other diagnostic categories.

When the isolation results in NSU contacts are examined in detail, it appears that the likelihood of a positive result depends on the clinical circumstances. Female contacts of men with recurrent NSU are less likely to have a chlamydial infection than contacts of men with first attacks of NSU, and primary contacts are more often positive than secondary ones. These results are consistent with those already reported in primary and secondary contacts of men with *Chlamydia*-positive and *Chlamydia*-negative NSU, from which it appears that perhaps one in three of patients at risk in fact develop a chlamydial infection (Oriel and others, 1972).

Chlamydial infection of the cervix was common in women with gonorrhoea, being present in sixteen out of 48 (33 per cent.) uncontaminated specimens from these patients. The significance of this association, which has also been found in men, has been disputed. Richmond and others (1972) have argued that a gonococcal infection may activate a genital chlamydial infection already present in a latent form. Were this so, it might be expected that trichomonal infection, which often causes an intense cervicitis, would do the same, but we found that only one of 25 women with this infection were *Chlamydia*-positive. It seems more likely that chlamydial and gonococcal infections may be sexually transmitted, either separately or together; multiple infections are, after all, not uncommon in patients with STD.

We were impressed by the rarity of chlamydial infection of women with no history of a contact with STD and with negative routine tests; in only one of 49 women in this category (2 per cent.) was *Chlamydia* found. As has been seen, control groups in the field of NSGI are difficult to define, but some observations by Fox (1973) are relevant; he has found that only one of fifty women in a general practice in London, who were sexually active but not promiscuous, were *Chlamydia*-positive. Of our 45 *Chlamydia*-positive women, 27 (60 per cent.) were taking an oral contraceptive; among the 202 *Chlamydia*-negative women, 75 (37 per cent.) were taking one of these agents. The

effect of these drugs on the susceptibility to STD is not known, although it has been said (Alexander, 1973) that gonococcal, chlamydial, herpesvirus, and cytomegalovirus infections are all commoner in women who are taking an oral contraceptive. We found that chlamydial isolates were more often obtained from women taking an oral contraceptive, but the differences were not statistically significant. This matter needs further study.

Chlamydial cervicitis is not, in our experience, distinctive clinically, although we were not able to use the operating microscope on such a large number of patients. Nevertheless, some abnormality of the cervix was present in 20 of 26 *Chlamydia*-positive women (excluding those with gonorrhoea or trichomoniasis), and cytological evidence of cervical inflammation was present in twelve. Among the eight *Chlamydia*-positive primary NSU contacts, cytology revealed evidence of inflammation in six, and we think that the value of cervical cytology in the investigation of NSGI may have been underestimated. In future work we will use it together with clinical observation of the cervix in the study of *Chlamydia*-positive and *Chlamydia*-negative women, matched by diagnosis and clinical circumstances as far as possible.

Of the 45 women found to have a chlamydial infection, only twenty (the NSU contacts) might normally have received a course of treatment with a tetracycline or similar antibiotic; in the remainder, the infection would, in all probability, have remained untreated. Thus a proportion of women attending a department of STD would be discharged in spite of the presence of chlamydial infection of the cervix. This situation is less likely to occur in men. Even if chlamydial isolations are not performed, men with NSU are usually treated with a tetracycline. Those with chlamydial infection associated with gonorrhoea are likely to develop post-gonococcal urethritis and be treated for this; in men without urethritis chlamydial infection is rare. We suggest that the value of culture for *Chlamydia* lies mostly in women, and that use of the technique should be considered whenever the investigation of possible genital infection is undertaken.

We encountered a high incidence of contaminated specimens. Out of 318 specimens, 71 could not be interpreted after culture owing to overgrowth by bacteria or yeasts. Of the 71 contaminated specimens, 26 came from women with candidosis, eleven from women with gonorrhoea, seven from women with gonorrhoea and trichomoniasis, and seven from women with trichomoniasis alone. Some modification of the antibiotics in the transport and growth media will be necessary; we are contemplating substituting gentamicin for streptomycin, an increase in the concentration of nystatin and possibly the introduction of metronidazole.

We believe that *Chlamydia* is a genital pathogen and causes a substantial portion of cases of NSU. If this is so, the discovery of genital chlamydial infection in women, unsuspected clinically and therefore untreated, becomes of importance. This investigation has shown that the screening of a large group of women for *Chlamydia* indicates that this situation exists among women attending a department of STD, who exhibit a pool of genital chlamydial infection. We are proposing to extend these investigations to other groups of women, both in departments of STD and elsewhere, and we think that results from these may be of interest in the study of the epidemiology of chlamydial genital infection. Epidemiological studies alone are, of course, not enough. Detailed clinical investigation of individual patients with chlamydial infection, such as those of Dunlop, Hare, Darougar, and Dwyer (1973), and additional study of serological responses to infection will also be necessary if the role of *Chlamydia* in NSGI is to be elucidated.

Summary

A series of 318 women, attending a department of sexually-transmitted diseases for the first time, had specimens collected from the cervix for culture for *Chlamydia* in irradiated McCoy cells. *Chlamydia* was isolated from 45 of 247 uncontaminated specimens, a recovery rate of 18 per cent. When the results were related to the diagnostic categories of the patients, it was found that chlamydial infection of the cervix was most commonly present in women who were sexual contacts of men with non-specific urethritis (isolates in 33 per cent.) and in women with gonorrhoea (isolates in 32 per cent.). *Chlamydia* was also found in association with other sexually-transmissible diseases (trichomoniasis, genital warts, genital herpes, and candidosis), but rarely in those with no history of contact with men with non-specific urethritis or gonorrhoea and with no clinical or microbiological evidence of sexually-transmitted disease.

It is suggested that cell culture tests for *Chlamydia* should be included when women are being investigated for possible genital infection.

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Infections chlamydiales du col utérin

SOMMAIRE

Chez une série de 318 femmes qui, pour la première fois, consultaient dans un Département de maladies sexuellement transmises, on préleva des spécimens cervicaux pour culture des *Chlamydia* sur cellules de McCoy irradiées. Les *Chlamydia* furent isolés chez 45 des 247 spécimens non contaminés, soit un taux de positivité de 18 pour cent. En confrontant les résultats selon les catégories diagnostiques, on trouva que l'infection chlamydiale du col était la plus fréquente chez les femmes partenaires sexuelles d'hommes atteints d'urétrite non spécifique (33 pour cent d'isolement) et chez les femmes atteintes de gonococcie (32 pour cent d'isolement). Les *Chlamydia* furent aussi trouvés en association avec d'autres maladies sexuellement transmises (trichomonase, végétations vénériennes, herpès génital et candidose), mais rarement chez les sujets n'ayant pas d'antécédents de contact avec des hommes atteints d'urétrite non spécifique ou de gonococcie et ne présentant pas d'évidence clinique ou microbiologique d'une maladie sexuellement transmise. On considère qu'il faudrait inclure les tests de culture sur cellules pour la recherche des *Chlamydia* dans les examens pratiqués chez les femmes chez lesquelles on recherche une infection génitale possible.